enzyme assays utilize the change in fluorescence which results when the phenolic hydroxyl becomes deacylated by the action of the enzyme (Page 50, lines 21-25).

Regarding phosphatase activity, many prior art references disclose the use of fluorogenic substrates to detect such activity. (*See e.g.*, Rotman, B. et al., "Fluorogenic substrates for Beta-D-galactosidases and phosphatases derived from flourescein (3,6-Dihydroxy fluoran), and its monomethyl ether," *Proc. Natl. Acad. Sci. USA*, 50, 1 (1963); Huschtscha, L.I., et al., "A rapid micro method for counting cells *in situ* using a fluorogenic alkaline phosphatase enzyme assay," *In Vitro Cell Dev. Biol.*, 25, 105 (1989); and Manafi M. et al., "fluorogenic and chromogenic substrates used in bacterial diagnostics," *Microbiol Rev.* 55, 335 (1991)). Phosphatase substrates that yield a resultant product, such as those identified in these references, are expected to be successfully employed in the methods of the present invention to detect phosphatase activity.

Regarding kinase activity, methods were known to a skilled artisan as of the filing date of the present invention, for detecting kinase activity within cells using fluorescence emissions to detect changes in ATP levels. Exhibit B describes how kinase activity effects ATP levels for both protein and non-protein substrates. Furthermore, Exhibit C provides examples of suitable ATP assays that were known as of the filing date of the present invention, that could be used to detect changes in ATP levels arising from kinase activity. Therefore, using fluorescent ATP assays, such as those discussed in Exhibit C, kinase activity can be detected according to the present invention.

Therefore, Applicants request reconsideration and withdrawal of the rejection of claim 21 for alleged lack of enablement under 35 USC §112, First Paragraph.

II. Double Patenting

Claims 19 and 25 stand rejected under the judicially created doctrine of double patenting over claims 22 and 23 of U.S. Patent No. 6,174,673. The Office Action alleges that the subject matter claimed in the instant application is fully disclosed in the patent and is covered by the patent since the patent and the application claim methods for identifying

bioactivity by high throughput screening comprising the same steps. Claims 19-24 and 26-45 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-5, 8-11, 15, 16, 18, 19, and 21 of U.S. Pat. No. 6,174,673. The Office Action acknowledged that the cited claims are not identical, but alleged that the conflicting claims are not patentably distinct from each other because pending claims of the present invention are drawn to a method of identifying bioactivity by high throughput screening comprising the same steps.

Applicants ask for a delay in responding to this double patenting rejection until the claims of the present application are found to be allowable.

In view of the amendments and the above remarks, it is submitted that the claims are in condition for allowance and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

Date: January 22, 2002

Lisa A. Haile, J.D., Ph.D.
Reg. No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

CUSTOMER NUMBER 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosures: Exhibit A
Exhibit B
Exhibit C

EXHIBIT A

MARKED-UP COPY OF THE SPECIFICATION AND THE CLAIMS SHOWING THE AMENDMENTS

In the Claims

Please amend the claims as follows:

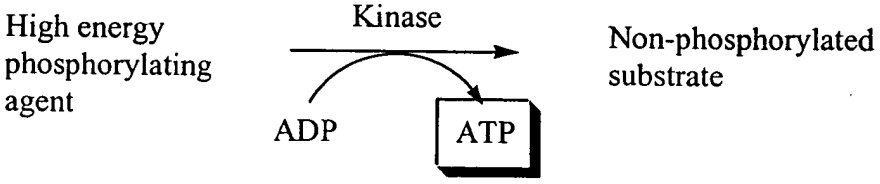
21. (Amended) The method of claim 20, wherein the enzymatic activity of interest is from an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, [glycosyl transferases,] phosphatases, kinases, [diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases,] monooxygenases, and acylases.

EXHIBIT B

Kinase Activity

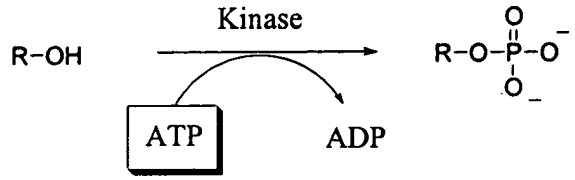
The ATP assay illustrated in the example below, for example, can be used to detect changes in ATP levels that result from kinase activity. This kinase activity includes activity for kinases that act on non-protein substrates, as well as kinases that act on protein substrates.

I. Kinases with non-protein substrates. Such kinases include, for example, pyruvate kinase, acetate kinase, creatine kinase, and phosphoglycerate kinase. In this case high energy phosphoryl donors, with higher phosphoryl group transfer potential than ATP, can be used to drive the reaction in the direction of ATP formation. Examples of such phosphorylating agents are phosphoenolpyruvate, acetyl phosphate, creatine phosphate, and 1,3-bisphosphoglycerate. (see for example: Cullis, P.M., Maxwell, A., and Weiner, D.P. Biochemistry **36**, 6059-6068, 1997 and references therein).



Fluorescent ATP assay

II. Protein kinases and other kinases using ATP as the high energy phosphoryl donor.



In this case a decrease in cellular ATP will be detected.

EXHIBIT C

Assays for ATP Based on Fluorescence

This Example provides illustrations of an ATP assay based on fluorescence that can be used to detect kinase activity according to the methods of the present invention.

Fluorescent phosphate assay.

$$ATP \xrightarrow{ATPase} ADP + Pi$$

$$Pi + m^7Guo \xrightarrow[\text{Phosphorylase}]{\text{Nucleoside}} \begin{matrix} m^7Gua \\ + \\ \text{ribose-1-phosphate} \end{matrix}$$

Note that the ATPase and nucleoside phosphorylase is cloned and expressed in the screening host. The expression of these detection enzymes can be tightly regulated such that they are only active when the kinase reaction is performed and measured thus minimizing deleterious effects on cell growth.